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Inactivation of *Aspergillus niger* spores from indoor air by photocatalytic filters

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ABSTRACT

The effects of UV-A and UV-C radiation on fungal spores were investigated before and after their germination in photocatalytic and non-photocatalytic air filters commonly used in heating, ventilating, and air conditioning (HVAC) systems.

Immediately after the coating of spores on filters, exposure to both types of UV radiation induced the appearance of an inactivation threshold for long durations of exposure probably resulting from the presence of *Aspergillus niger* spores inside the activated charcoal layer. The use of a thin photocatalytic filter without activated charcoal demonstrated a better disinfection efficiency with total inactivation of the spores, due to an optimal contact between spores and TiO_2 coating.

The effects of UV radiation were then assessed on spore germination for both types of filters. The inactivation of spores in illuminated photocatalytic filters resulted in an irreversible inhibition of the fungal germination under UV-A or UV-C radiation. In contrast, fungal spores were able to germinate in non-photocatalytic filters despite previous exposure to both types of UV radiation. The monitoring of ergosterol amounts, the major sterol of fungal membranes, corroborated these results.

Finally, UV-A or UV-C radiation exposure of filters after spore germination had a lesser disinfection efficiency than experiments whereby spores had just been applied onto the filters, due to the absence of contact between the biological pollutants and the photocatalyst coating.

Our results thus demonstrated the interest to use photocatalytic filters ensuring optimal contact between pollutants and TiO_2 coating to lead to a total inactivation of fungal spores in filters of HVAC systems.

1. Introduction

Today, populations in developed countries spend more than 80% of their time in confined indoor environments. Indoor air quality (IAQ) and the potential impacts of indoor air contamination have therefore become of particular concern for public health and safety reasons. Factors affecting indoor environments mainly include relative humidity, temperature, air exchange rate, air movement, ventilation, chemical (gaseous or particles) and biological pollutants (bioaerosols). Increased bioaerosol levels in indoor environments may come from various sources including building materials when sufficient moisture and nutrients are combined or air conditioning or ventilation systems under specific conditions [1–6].

General interest in exposure to bioaerosols has developed over the last two decades, because of their presence in occupational environments and their serious implications on human health. Indeed, many studies have shown a link between the presence of bioaerosols indoors and adverse health issues including infectious diseases, respiratory pathologies, allergic reactions [7,8]. Moreover, many of the medical symptoms due to exposure to biological pollutants are accentuated in confined environments due to the accumulation of specific bioaerosols as a result of poor building ventilation.

HVAC systems are commonly used in residential, commercial or industrial buildings to maintain indoor air quality and ambient temperature. Ventilation allows the removal of interior airborne pollutants through filtration, cooling or heating fresh indoor air at

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the same time. In HVAC systems, traditional filters usually allow the capture of biological particles but do not kill them. Eventually, due to favourable conditions such as high humidity, poor design or maintenance or accumulation of organic compounds collected by filters as source of nutrients, aerosolized microorganisms become able to survive and grow within these filters [6,9–11]. HVAC systems can therefore disseminate airborne microorganisms, toxins, allergens and microbial volatile organic compounds (MCOVs) from contaminated filters into the environment where they are inhaled by building occupants [12–15]. In addition, the accumulation of microorganisms on the filters leads to a decrease in their efficiency to remove pollutants in the long run.

In this context, the recent emergence within the HVAC industry of the photocatalytic process presents a great opportunity to address contamination of air filters concerns. The effectiveness of photocatalysis to inactivate a wide range of harmful microorganisms has been documented in the literature for a number of years [16–22]. If dimensioned appropriately, photocatalysis may represent an effective process adaptable to a number of applications for disinfection of both indoor air and drinking water in various environments such as industrial and health care environments [23,24]. Within the HVAC industry, the photocatalytic process is still an emerging technology, in particular where photocatalytic filters are employed in the purge of airborne microorganisms. Moreover, very few studies have taken an interest in the survival of aerosolized microorganisms trapped into photocatalytic filters [20,22,25].

The main contribution of this study is to compare the efficiency of commercial photocatalytic and non-photocatalytic filters under UV-A or UV-C radiation to inactivate *Aspergillus niger* (*A. niger*) spores. Fungal spores are commonly detected in indoor environments as well as in filters of ventilation systems [26–28]. Moreover, *A. niger* strains are known for their resistance to any environmental stress conditions, and especially their spores which contain aspergillin, the black fungal spore pigment of *A. niger*, protecting them from UV radiation exposure [29,30]. As a result, they can be considered as representative airborne microorganisms appropriate for the assessment of the efficiency of photocatalytic filters [22,31,32]. In view of this, we investigated the effects of UV radiation exposure: (i) on *A. niger* spores immediately after their coating on filters; (ii) on fungal germination after exposure to UV radiation in filters; (iii) on *A. niger* spores after their germination in filters. To go further in our investigation, we also performed chemical analyses to monitor the amount of ergosterol, the principal sterol in fungal membranes, as a chemical marker of the quantity of both spores and mycelia in filters after UV radiation exposure.

2. Experimental conditions

2.1. Fungal strain and culture conditions

For all experiments, the *A. niger* strain IP 1187.79 was used and maintained on malt extract agar (MAEc medium) supplemented with chloramphenicol to limit bacterial growth (0.5 g/l). Colonies of *A. niger* were grown on MEAc agar plates for 7 days in a climate chamber where temperature and relative humidity (RH) were fixed at 25 °C and 98%, respectively.

2.2. Culture media

An aqueous solution with chloramphenicol was used to recover spores from *A. niger* colonies grown on MEAc agar plates.

A rich medium appropriate for an optimal growth of fungi was used to spray the spores on filters. It was composed of 1 g of KH_2PO_4, 0.5 g of $MgSO_4$, $7H_2O$, 5 g of pancreatic digest of casein, 10 g of glucose, 220 g of glycerol, 0.1 g of chloramphenicol per litre of demineralized water, pH 5.4–5.7.

2.3. Preparation of the initial suspension of A. niger spores

Spores were washed from 7-day MAEc agar plates and then counted in a Thoma counting chamber with an optical microscope (Zeiss Axio ScopeA1 model). The spore concentration was finally adjusted with appropriate dilutions to 2×10^7 spores/ml.

2.4. Light sources

Experiments under UV radiation were performed using two Philips PL-L mercury lamps (18 W), one emitting only UV-A radiation with a peak emission at 365 nm and the other emitting only UV-C radiation at 254 nm. A digital radiometer (VLX-3W, UVItec) was used to determine the radiance intensity of both light sources. It was equipped with 365 nm and 254 nm detectors calibrated in the spectral range of 355–375 nm and 254 nm, respectively. The total radiance intensity at the surface of the filters was of 3.6 mW/cm^2 for UV-A experiments and of 3.35 mW/cm^2 for UV-C experiments. A good homogeneity of the radiance intensity was confirmed on the whole surface of filters samples used for all experiments.

2.5. Filters

We tested three non-woven filter materials supplied by the manufacturer Ahlstrom Corp. Two filters were 2.5 mm thick, multilayered materials, used as gas filters for general ventilation. They were both composed of an inner activated charcoal layer supplied in diameters ranging from 0.25 to 0.60 mm, between two layers of non-woven fibres made of cellulose and polyester. These two filters were named non-photocatalytic AC filter and photocatalytic AC filter for this study. The photocatalytic filter was coated with PC500 TiO_2 by Cristal Global (anatase > 99%, specific surface area: 350–400 m^2/g, crystallites mean size of about 5–10 nm). The TiO_2 coating procedure was developed by the Ahlstrom firm (1069950A1 European patent). Fig. 1 shows the scanning electron microscopic pictures of a photocatalytic AC filter with TiO_2 coating. Accordingly, the diameter of the filter fibres was about 20 μm.

The third filter, called thin photocatalytic filter for this study, 0.76 mm thick, was made of cellulosic and polyester fibres with a PC500 TiO_2 coating but without the activated charcoal layer. For all experiments, filters were cut into pieces of 5 cm × 5 cm.

2.6. Procedures

2.6.1. Controlled coating of the spores on filters

An Iwata Airbrush Eclipse HP-BCS set up with a compressor was used to spray the *A. niger* spores suspension onto the filters. The suspension was sprayed with an airflow rate adjusted to 8.6 ml/min. The suspension was stirred every three tests to ensure a uniform distribution of spores in the suspension and no spore agglomeration, before spraying. The whole device was located under a class II microbiological safety cabinet (MSC) to ensure sterility of the experiments. This initial quantity of fungal spores coated on each filter was determined to be of 5×10^6 spores, after an extraction procedure, by dilution plating on MEAc medium and incubation for 48 h at 25 °C and 98% RH, before counting *A. niger* colonies.

2.6.2. UV radiation exposure

The effects of UV-A (3.6 mW/cm^2) or UV-C radiation (3.35 mW/cm^2) exposure of non-photocatalytic or photocatalytic filters were investigated:

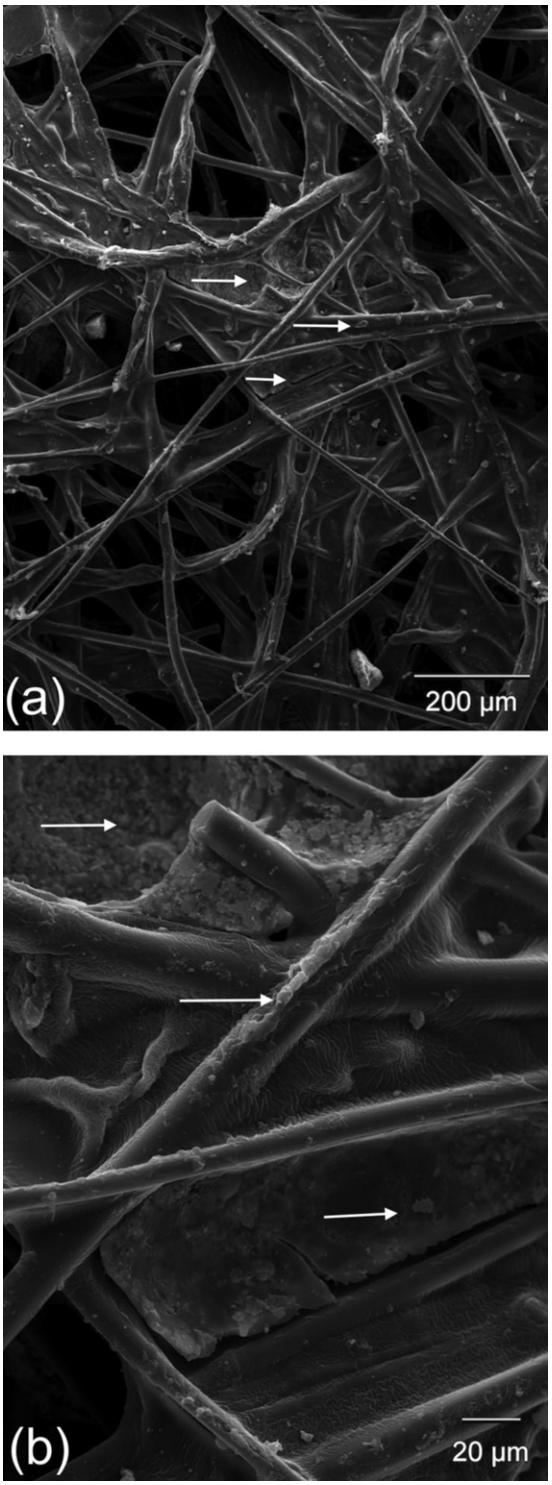

Fig. 1. (a and b) Scanning electron microscopic pictures of the outer layer of a photocatalytic AC filter. White narrows indicate the presence of TiO_2 photocatalyst (FEI ESEM model XL 30 scanning electron microscope).

(1) On the viability of fungal spores: after spore coating, filters were exposed to UV radiation with duration ranging from 2 to 17 h and spores were then immediately extracted.
(2) On spore germination: for this series of experiments, an initial quantity of approximately 5×10^6 fungal spores was coated on filters and exposed to 4 h of UV-A radiation and of 2 h of UV-C radiation. Then filters were incubated under optimal conditions for spore germination into a climatic chamber in the dark, at 25 °C under 98% relative humidity, for 8 days, before the extraction procedure,
(3) On the viability of fungal spores after their germination in filters: an initial quantity of approximately 5×10^6 spores was coated on filters, then incubated under optimal conditions for spore germination at 25 °C under 98% relative humidity. After 8 days of incubation, filters were finally exposed to 4 h of UV-A or 2 h of UV-C radiation, before the extraction of spores.

These different approaches are presented in Fig. 2.

UV radiation experiments were carried out within an incubator where temperature of 22 °C and relative humidity of 45% were controlled with a thermo-hygrometer throughout the exposure to UV radiation. These values correspond to the real parameters of HVAC systems. Filters were placed within closed sterile quartz Petri dishes during UV radiation exposure to ensure the sterility of the experiments. Durations of exposure to UV-A or UV-C radiation ranged from 2 to 17 h. Dark control experiments with filters contaminated within the same experimental conditions but without UV light exposure were carried out at the same time (Fig. 2). Two replicate filters were used for each duration of exposure to UV or dark conditions. The quantity of viable spores was then evaluated, after an extraction procedure, by dilution plating on MEAc medium and incubation 48 h at 25 °C with 98% RH. Experiments were performed in triplicate to ensure reproducibility.

2.6.3. Spore extraction procedure

Spores were extracted from the filters by disintegration in an aqueous suspension containing 0.1% Tween 80 v/v, pH 7. The concentration of fungal spores was then determined by dilution plating on MEAc agar plates and incubation at 25 °C and 98% RH for 48 h, before colony counting. A series of experiments revealed that this method had no deleterious effects on the viability of fungal spores. After the extraction procedure, the ratio of recovered spores was $83 \pm 2\%$ for the photocatalytic AC filters and $89 \pm 4\%$ for the photocatalytic AC filters. The extraction ratio was of $95 \pm 2\%$ for the thin photocatalytic filters. These results revealed a good efficiency to remove almost all of the coated microorganisms.

2.6.4. Ergosterol extraction

The procedure used for an efficient extraction of free and esterified ergosterol was based upon that developed by Brodie et al. [33]. Filters with *A. niger* spores were suspended in 25 ml of methanol to extract free ergosterol. The ester was saponified by adding 5 ml of KOH in ethanol (80 g/l). The whole mixture was stirred for 30 s by vortex and for an additional 60 s of sonication (Vibra-Cell Sonics & Materials, 20 kHz). The samples were then heated at 85 °C for 30 min to facilitate the saponification. After cooling at room temperature, aqueous methanol solutions were extracted with 10 ml of hexane and 2 ml of water. The samples were centrifuged three times at $3000 \times g$ for 3 min. The hexane phases were pooled and evaporated. Samples were finally dissolved in 1 ml of methanol and filtered (0.45 µm) for HPLC analysis.

2.7. HPLC analysis

HPLC analysis of ergosterol extracts was carried out with an Agilent Infinity 1290 high performance liquid chromatograph (HPLC) system equipped with a reverse phase column (Agilent Superspher 100 RP-18, 4 µm, 4 × 125 mm) and a Diode Array Detector (Varian Prostar 330 PDA) set at 282 nm for ergosterol detection. Elution was performed at a flow rate of 1 ml/min with methanol as the mobile phase. The ergosterol concentrations were calculated based on a standard curve obtained from ergosterol solutions of 0–22 mg/l.

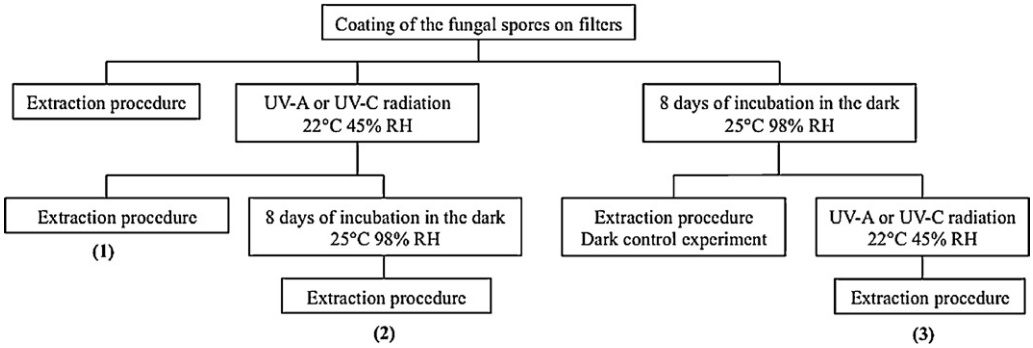

Fig. 2. Experimental procedure to evaluate the effects of UV radiation on fungal spores coated on non-photocatalytic or photocatalytic filters.

3. Results and discussion

3.1. Study of the viability of fungal spores within filters in the dark

A series of experiments were carried out in order to investigate the ability of fungal spores to survive and germinate inside filters in the dark. First, *A. niger* spores in suspension in rich liquid medium were sprayed on non-photocatalytic and photocatalytic AC filters. Fig. 3 shows the number of fungal spores extracted from each filter after different durations of contact in the dark under optimal incubation conditions (25 °C, 98% RH).

We first observed that the initial quantity of fungal spores coated on filters was similar from one test to another and equal to 5.0×10^6 spores with a standard error of 4%. Evaluation of the number of spores extracted from quarters of filters also revealed a good dispersion of the spores over the surface of the filters. This result was confirmed by the macroscopic observation of the surface of filters after 8 days of incubation under optimal growth conditions (Fig. 4(a)). We observed in Fig. 4(a) a good distribution of the conidial heads over the entire surface of the filter showing the germination of the fungal spores. Thus, our method allowed the development of coating with a replicable number of spores between the tests and a homogenous spread of spores over the whole surface of the filters. This is a critical point to rigorously assess the disinfection efficiency of every type of photocatalytic material.

Moreover, the potential deleterious effect of the extraction step on microorganism viability was also evaluated by applying the procedure to the initial fungal spores suspension. No change in spore concentration was observed before and after the extraction procedure, thus demonstrating no damages on spore viability.

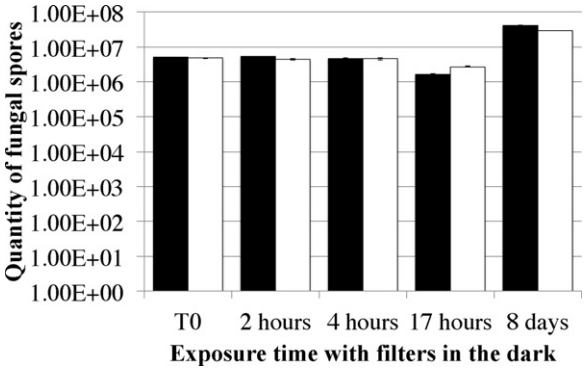

Fig. 3. Monitoring of the quantity of viable *A. niger* spores extracted from non-photocatalytic (■) or photocatalytic AC filters (□) after different periods of contact in the dark at 25 °C with 98% relative humidity.

After 2 or 4 h of contact in the dark, no modification in viable fungal spores quantity from either type of filter was observed (Fig. 3). In comparison, we noticed a slight decrease after 17 h, probably resulting from the presence of germinating spores inside filters, not capable of growing on MAEC agar plates. The increase of approximately 1 log of the spore quantity after 8 days of contact with both filters corresponded to the typical growth of fungal mycelia resulting from spore germination. Additionally, direct examination of filters (Fig. 4(b)) under an optical microscope after 8 days of incubation was particularly useful for the observation of conidial heads and also hyphae (long branching filamentous structure of fungi), two key points that confirm the spore germination inside the photocatalytic filter in the dark. *A. niger* spores of

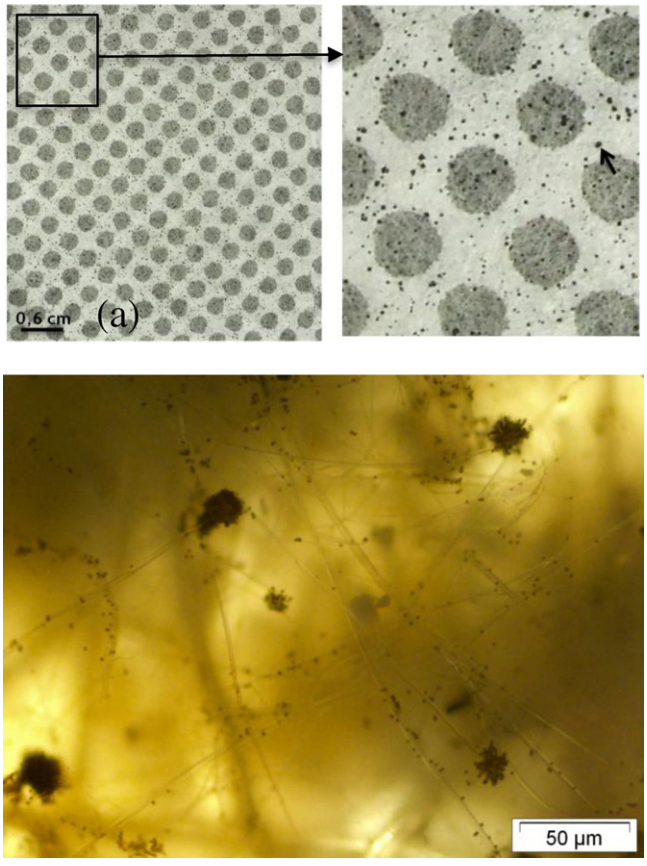

Fig. 4. (a) Picture of the surface of a photocatalytic AC filter after coating of fungal spores and 8 days of incubation in the dark at 25 °C and 98% RH. The small black dots correspond to the conidial heads. (b) Picture from an Olympus microscope BX51 model of the inside of a photocatalytic AC filter after the coating of fungal spores and 8 days of incubation in the dark at 25 °C and 98% RH.

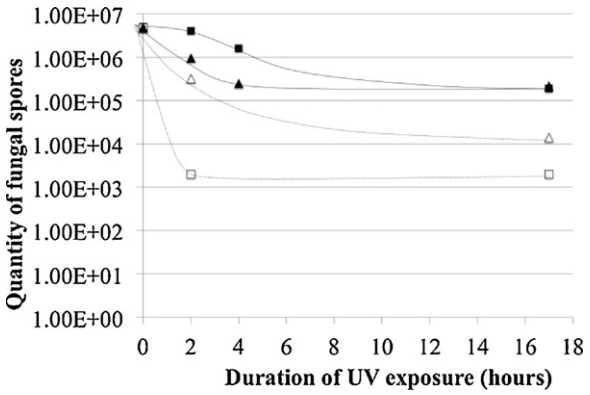

Fig. 5. Monitoring of the quantity of viable fungal spores extracted from: non-photocatalytic AC filters after different durations of exposure to UV-A (■) (3.6 mW/cm^2) or UV-C radiation (□) (3.35 mW/cm^2); photocatalytic AC filters after different durations of exposure to UV-A (▲) (3.6 mW/cm^2) or UV-C (△) radiation (3.35 mW/cm^2).

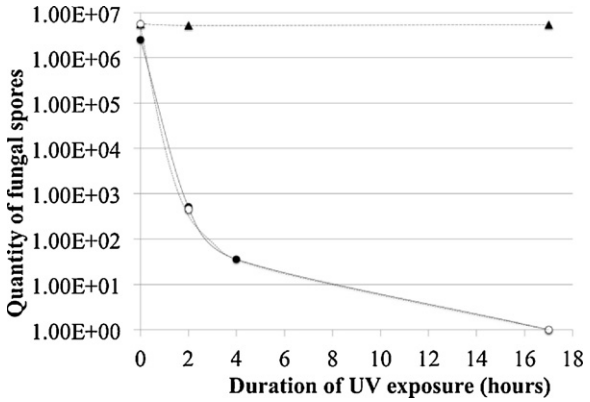

Fig. 6. Monitoring of the quantity of viable fungal spores extracted from thin photocatalytic filters without activated charcoal: not exposed to UV radiation (▲); exposed to UV-A (●) (3.6 mW/cm^2) or UV-C radiation (○) (3.35 mW/cm^2).

approximately 4 μm in diameter, with their dark pigment colour, were also visible on the filter fibres. Picture in Fig. 4 was representative of the all filter samples observed. These results demonstrated the ability of *A. niger* fungi to grow inside photocatalytic or non-photocatalytic AC filter in presence of nutrients from the rich liquid medium.

Subsequently, *A. niger* spores in suspension in a minimal medium without organic carbon source were sprayed on both types of filters but no germination was observed. Indeed, neither a decline nor an increase in the number of spores was observed, even after 30 days of contact in the dark under the same experimental conditions. We can conclude that none of the components of either filter can be used by spores as source of nutrients for their germination. Previous studies also described this phenomenon and authors made the hypothesis that the chemical composition of the binder for the filters could influence the rate and strains of fungal colonization [34].

However, detection of viable spores in filters after a 30-day period indicated that they were able to remain inside filters in the dark after a long exposure. Under realistic environmental conditions, with optimal humidity or accumulation of dusts on filters as nutrients sources, we can make the hypothesis that some of these spores would be able to germinate and to initiate the formation of fungal mycelia able to colonize the filter of HVAC systems.

3.2. Effects of UV-A or UV-C radiation exposure on A. niger spores in filters

3.2.1. Effect on the viability of the spores

First of all, a series of experiments were carried out in order to assess the effects of UV-A or UV-C radiation on spores immediately after their coating on both photocatalytic and non-photocatalytic AC filters. *A. niger* spores were coated on filters in suspension in a liquid rich medium.

The quantities of spores extracted from each type of filters after different durations of exposure to UV-A or UV-C radiation are presented in the graph of Fig. 5.

3.2.1.1. Exposure to UV-A radiation. The number of spores in photocatalytic AC filters started decreasing from 2 h of UV-A radiation exposure (▲) while a longer exposure of 4 h was necessary to induce a decrease in non-photocatalytic AC filters (■). The faster inactivation of *A. niger* spores in photocatalytic filters for short exposures to UV-A radiation can be explained by the combined action of UV and TiO_2 photocatalyst coating. However, after 17 h of UV-A radiation, numerous spores extracted from both types of filters were still viable. The quantities inside both filters were very similar (approximately 2×10^5 spores) and did not evolve so much in comparison with that numerated from the previous exposure time, thus indicating an inactivation threshold for the spores under UV-A radiation.

3.2.1.2. Exposure to UV-C radiation. When filters were exposed to UV-C radiation, the quantity of fungal spores decreased more rapidly in the case of non-photocatalytic AC filters. We can make the hypothesis that the TiO_2 coated at the surface of the photocatalytic AC filter absorbed a significant part of UV-C radiation, which therefore were not able to reach *A. niger* spores and damage them. After 17 h of UV-C radiation exposure, we also noticed an inactivation threshold.

In order to better understand the implication of the activated charcoal in the appearance of this inactivation threshold, we carried out a series of experiments with thin photocatalytic filters without activated charcoal layer. Thus, spores were coated on these thin photocatalytic filters and exposed to UV-A or UV-C radiation within the same experimental conditions. Results are presented in the graph of Fig. 6.

The decrease of the number of fungal spores is very similar during UV-A or UV-C photocatalytic experiments. Moreover, in comparison with AC filters, we noticed a faster inactivation kinetic of the microorganisms and no viable spore was detected after 17 h of exposure to both types of UV radiation. As a result, we suggested that the spores still viable in AC filters after long exposure times were those retained inside the inner activated charcoal layer. They were thus protected from UV radiation or not exposed to a sufficient dose to be definitely inactivated. Moreover, they were not in direct contact with the photocatalytic coating at the surface of the photocatalytic filter, which is a crucial point for the disinfection efficiency of the photocatalytic process [23]. In contrast, for the thin photocatalytic filter, we observed a better inactivation of the microorganisms, as their contact with the photocatalyst coating was closer.

3.2.2. Effect on spore germination

Our objective was then to investigate whether the inactivation of the microorganisms described above (Fig. 5) was irreversible after their exposure to UV radiation (non-photocatalytic AC filter) or to UV radiation combined to titanium coating (photocatalytic AC filter). Therefore, we investigated the ability of fungal spores to germinate and develop a mycelium in filters after exposure to UV-A or UV-C radiation. Control experiments with contaminated filters were performed within the same experimental conditions but without UV exposure. Results are presented in Fig. 7.

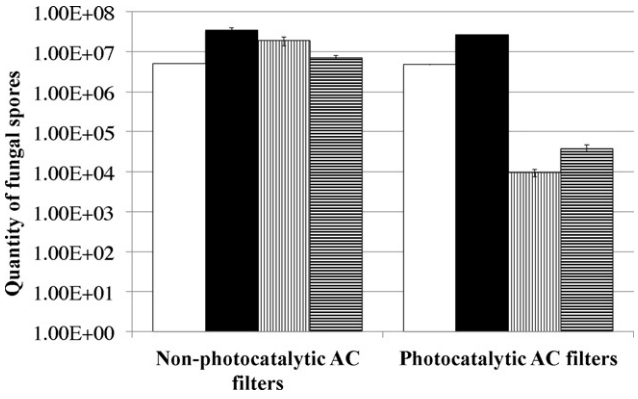

Fig. 7. Quantities of viable fungal spores extracted from non-photocatalytic or photocatalytic AC filters: immediately after their coating (□) or after 4 h of exposure to UV-A radiation (3.6 mW/cm^2) (▥) or 2 h of exposure to UV-C radiation (3.35 mW/cm^2) (▤) and then 8 days of incubation in the dark (25 °C and 98% RH). Control experiment (■) corresponds to filters incubated in the dark under the same incubation conditions but without UV exposure.

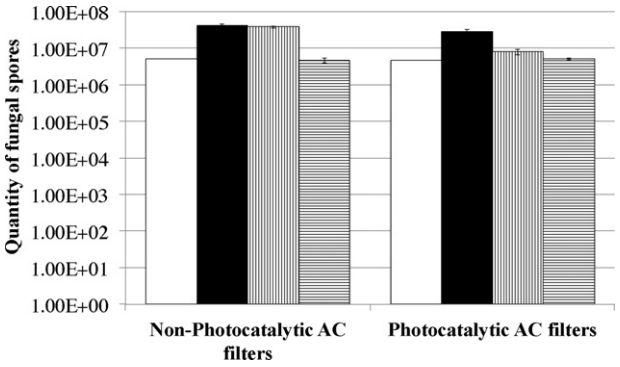

Fig. 8. Quantities of viable fungal spores extracted from non-photocatalytic or photocatalytic AC filters: immediately after their coating (□) or after their incubation for 8 days in the dark (25 °C and 98% RH) and then 4 h of UV-A radiation (3.6 mW/cm^2) (▥) or 2 h of UV-C radiation (3.35 mW/cm^2) (▤). Control experiment (■) corresponds to filters incubated in the dark under the same incubation conditions but without UV exposure.

Concerning the control filters not exposed to UV radiation, we observed an increase in the number of viable spores of approximately 1 log, which corresponds to a normal growth of a fungal mycelium after 8 days of incubation with optimal temperature and humidity conditions. For non-photocatalytic AC filters, 4 h of UV-A or 2 h of exposure to UV-C radiation induced only a slight deleterious effect on the spore germination. Indeed, the quantities of spores extracted from these filters are fairly similar to that extracted from dark control filters. In comparison, the quantity of spores extracted from photocatalytic filters is clearly lower after the same duration of UV exposure. These results are surprising given previous results of Fig. 5(■) and (□) that indicated a significant inactivation of the spores in non-photocatalytic AC filters immediately after 4 h of UV-A or 2 h of UV-C light. Moreover the quantity of damaged spores was more important in non-photocatalytic AC filters than in photocatalytic filters, after 2 h of UV-C radiation (Fig. 5(▲) and (△)). Our results clearly demonstrated that *A. niger* spores are not able to germinate in photocatalytic AC filters after exposure to UV radiation. In contrast, the initial deleterious effects of UV radiation in absence of TiO_2 coating seem to be reversible. Indeed, we observed an increase of the number of spores during the dark incubation period for the non-photocatalytic filters. We then controlled that the inhibition of spore germination was undoubtedly the consequence of their inactivation by the illuminated TiO_2 coating rather than the effect of a lack of nutrients, potentially destructed by the photocatalytic process. Therefore, experiments within the same experimental conditions were conducted with the addition of rich liquid medium on photocatalytic AC filters before the 8-day incubation period in the dark in order to eventually allow spores to germinate. However, no spore germination was observed. Thus, the photocatalytic process induced an inhibition of the spore germination and permanently damaged their metabolism. Without the photocatalyst coating, spores would probably be able to colonize AC filters in spite of UV exposure.

The measurement of the ergosterol amount present in both types of filters confirmed these results (Table 1). Ergosterol is the major sterol component of membranes in mycelia, spores, and also vegetative cells, widely used as an estimate of fungal biomass in various environments [35,36].

Table 1 shows a decrease of 95% of ergosterol amount in photocatalytic AC filters after 4 h of UV-A exposure and 8 days of incubation in the dark, contrary to non-photocatalytic filters. However, concerning UV-C radiation exposure, we noticed that the ergosterol amounts extracted from both types of filters are very close. *A. niger* spores contain in their membrane aspergillin, a black pigment that protects them from UV radiation. As a result, we can formulate the hypothesis that UV-C radiation could have a mutagenic action on fungal spores rather than a lethal action, which would cause membrane function damages and alterations in the ergosterol biosynthetic pathway without limiting spore germination [37]. This could explain the difference between the quantity of spores and the amount of ergosterol extracted from photocatalytic AC filters after UV-C radiation exposure and incubation in optimal conditions.

3.2.3. *Effects on spores after their germination in filters*

Our last objective was to investigate the disinfection efficiency of both types of filters under UV radiation after their colonization by *A. niger* fungi. Series of experiments were thus carried out in order to evaluate the effects of UV-A or UV-C radiation on spores in non-photocatalytic or photocatalytic filters, after their germination under optimal growth conditions during 8 days in the dark. The quantity of fungal spores extracted from each type of filter is presented in Fig. 8.

Results of Fig. 8 indicate that for non-photocatalytic AC filters, only 2 h of UV-C radiation induced a slight decrease of 1 log in the number of spores. For photocatalytic AC filters, UV-A or UV-C radiation exposure had an effect on the spores 8 days after their growth less important than when they had just been applied onto the filters. Moreover, no difference in ergosterol amounts extracted from each filter was noticed in the dark after UV-A or UV-C radiation exposure (Table 1). Macroscopic observations of the filters after 8 days of incubation in optimal growth conditions revealed the development of hyphae and conidial heads above the whole surface of the filters (Fig. 9).

As fungal structures and in particular spores located on conidial heads were not in direct contact with the TiO_2 coating during UV radiation exposure, reactive oxygen species created at the surface of TiO_2 were not able to inactivate them. This observation corroborated previous results reporting that the contact between TiO_2 and microorganisms is necessary for the efficiency of the photocatalytic process [23,38]. However, for non-photocatalytic filters,

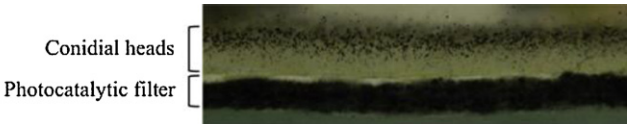

Fig. 9. Picture of conidial heads developed above the surface of a photocatalytic filter after coating of the fungal spores and 8 days of incubation in optimal growth conditions (25 °C, 98% RH).

Table 1
Percentage of ergosterol extracted from non-photocatalytic or photocatalytic AC filters with or without exposure to UV radiation prior to their incubation in the dark at 25 °C under relative humidity of 98%.

Type of filter	Percentage of ergosterol (%)		
	8 days of incubation in the dark without UV exposure	Incubation in the dark after 4 h of UV-A radiation	Incubation in the dark after 2 h of UV-C radiation
Photocatalytic	100	5	14
Non-photocatalytic	100	95	22

we noticed that the quantity of spores initially coated is close to the one extracted after UV-A or UV-C radiation exposure. This indicated that UV photochemistry did not induce deleterious effects on *A. niger* spores where they are released into the air.

4. Conclusions

The inactivation of spores in both types of filters immediately after their coating required long durations of exposure to UV-A or UV-C radiation. Moreover, the presence of activated charcoal within photocatalytic or non-photocatalytic filters induced the appearance of a threshold probably resulting from the persistence of *A. niger* spores inside the thick inner layer of activated charcoal. Therefore, our results highlighted the difficulties to combine the adsorption properties of activated charcoal and the microbicidal action of photocatalysis.

However, a thin photocatalytic filter without activated charcoal induced total inactivation of spores under UV radiation, thus demonstrating the interest to use photocatalytic filters to ensure optimal contact between pollutants and TiO_2 coating, for indoor air disinfection.

Moreover, the inactivation of spores in photocatalytic filters under UV-A or UV-C radiation resulted in an inhibition of their ability to germinate. The irreversible effect of the photocatalytic process on fungal spores and on their metabolism was thus demonstrated, in contrast with photochemistry. However, the effect of photocatalytic filters exposed to UV-A or UV-C radiation on the spores was less important after their growth and the colonization of the filters by fungal mycelia, than right after the spores had just been applied on the filters. As a result, for an optimal use of photocatalytic filters in real operating environment of HVAC systems, UV exposure of photocatalytic filters from the beginning of the systems seems to be essential in order to avoid microbial colonization.

For further research, the development of a new generation of photocatalytic filters with high filtration efficiency, thus improving the contact between microorganisms and TiO_2, should be considered. Moreover, many questions remain regarding the release of chemical by-products resulting from the inactivation of microorganisms. Considering previous results about the photocatalytic inactivation of bacteria in suspension in water, the identification of potential toxic by-products generated during the inactivation of bioaerosols in photocatalytic filters is a critical point [39]. Indeed, any indoor air disinfection technology must be matched with an optimal dimensional approach without inducing the formation of either toxic intermediates or resistant microorganisms.

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